

# The Use of Cation -Exchange Resins in Natural Water Trace Metals Research

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## Preface

This text was originally submitted as the literature review component for my MChem degree in February 2005. This version is largely to address the comments that were provided by Dr John Hamilton-Taylor on the submitted text and to correct some errors that were spotted after submission. In addition the section on the DGT technique has been amended to remove the unnecessary section on soil and sediment uses and to include a new brief overview of other DGT cation binding phases used.

A Stockdale. July 2005

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**Abstract-** The desire to measure low metal concentrations in natural waters stems principally from the aim to understand the effects of trace metals on aquatic biota and to understand the fate of metal pollutants. Trace metals rarely exist in the free ion form, with distribution of the different species being dependent upon pH,  $E_h$ , and the types of organic/inorganic ligands and colloidal surfaces. Use of cation exchange resins, particularly Chelex-100, has been developed over the past few decades and enables preconcentration to yield, upon elution, measurable concentrations of trace metals. These resins can also yield data on the speciation (by measuring lability) of a metal in solution by adjusting the contact time of the sample with the resin. Several speciation schemes have been developed where separation techniques (such as dialysis) are coupled with Chelex-column methods (short contact time) and Chelex-batch methods (long contact time). With the exception of recent equilibrium based studies Chelex is always used in an environment where the number of binding sites is in large excess compared to the number of competing cations, meaning competition effect are not significant, although kinetic factors are important in Chelex lability studies. Nitric acid elution generally yield good recovery rates (>90%) and stepwise elution has been shown to be the most effective for both metal recovery and minimising the eluent volume. The development of DGT speciation studies may bring about a technique that is less complex but easier to undertake and yields simple yet useful complexation data. The potential for IDA-resins to yield free-ion concentrations based on equilibrium-based sorption is a beneficial development that is currently being investigated. This review investigates the use of cation exchange resins by assessing the resin properties, the techniques developed and the optimum sorption conditions.

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**Keywords:** ion-exchange resin, Chelex, DGT binding phases, trace metals, speciation

**Contents**

Part 1	Introduction	P4
Part 2	Properties of Chelex	
	2.1 Basic resin properties	P8
	2.2 Stability constants of metal-resin complexes	P11
Part 3	Chelex column methods	P14
Part 4	Speciation studies utilising Chelex	
	4.1 Chelex batch methods	P19
	4.2 Speciation schemes	P20
Part 5	Recent developments	
	5.1 Diffusion gradients in thin-films	P26
	5.2 The equilibrium based sampler	P29
Part 6	Summary and proposal for further work	P31
	Acknowledgements	P33
	References	P33
	Appendix 1. Pre-treatment of samples for Chelex column studies	P37

## **Tables and Figures**

### *Tables*

1	Examples of chemical species	P4
2	Resins and their functional groups	P6
3	Selected parameters in several Chelex column studies	P14
4	Preferred elution reagents for selected metals	P18

### *Figures*

1	The basic structure of the styrene divinylbenzene copolymer	P8
2	Change in structure of resin functional groups with pH	P8
3	Theoretical titration of IDA	P9
4	Comparisons of stability constants of IDA with various compounds	P13
5	Binding spectrum for soluble trace metal species	P20
6	The Figura & McDuffie trace metal speciation scheme	P21
7	The Florence & Batley trace metal speciation scheme	P22
8	The Chakrabarti <i>et al</i> trace metal speciation scheme	P24
9	Representation of the free ion concentration in a DGT assembly	P26

## Part 1 Introduction

Chemical speciation refers to the form of ions in solution and their interactions with other constituents, both aqueous and solid. The principal purpose of studying metal speciation in natural waters relates to determining their relative nutrient or toxic effects with respect to biota, and to understand the behaviour of metal pollutants in the aquatic environment. It is important therefore, to consider all forms of metal species in the system (Batley, 1983).

Many trace metal species are observed, the distribution between the possible forms being dependent upon the type and concentration of the trace metal, pH,  $E_h$  and types of organic and inorganic ligands, and colloidal surfaces (Hart & Davis, 1981). Some metals have been shown to exhibit almost complete complexation in surface waters (e.g. Cu(II) in organic complex), whereas others (e.g. Mn(II)) show negligible organic complexation (Hering & Morel, 1990). The toxicity of a given trace metal may be mitigated by complexation, notably with dissolved and particulate organic matter, subsequently offsetting toxicological impacts of many trace metals for a given concentration (McNee & Martin, 1999). In order to fully understand the potential effects on biota the interactions between the individual species must be understood, only then can the complete bioavailability be assessed. The lability, i.e. the rate at which complexation/competition kinetics occurs also requires consideration.

### Species types

Table 1 gives examples of some key species types.

Table 1. Examples of chemical species.

Species	Examples of	
	Labile species	Non-labile species
Inorganic <sup>1</sup>	$[Zn(H_2O)_6]^{2+}$ , CuOH <sup>+</sup> , CdCl <sub>4</sub> <sup>2-</sup>	ZnCO <sub>3</sub>
Organic <sup>1</sup>	Cu-glycinate	Cd-cysteinate
Surface adsorbed onto inorganics <sup>1</sup>	Pb-Fe <sub>2</sub> O <sub>3</sub>	Pb-MnO <sub>2</sub>
Surface adsorbed onto organics	Cu-humic acid <sup>2</sup>	Zn-tannic acid
Colloidal <sup>3</sup>	Fe <sub>2</sub> O <sub>3</sub> , SiO <sub>2</sub>	
Lipid-soluble metal complexes <sup>4</sup>	Alkylmercury compounds	

<sup>1</sup>Examples from Florence (1977). <sup>2</sup>Humic complexes may be labile or non-labile. <sup>3</sup>These complexes may be non-labile under stable conditions and may have surface-adsorbed metal-ions. <sup>4</sup>Florence (1989) recognises that total metal analysis alone will not predict toxicity and proposes a scheme that includes lipid-soluble complex determination.

Until the aspiration of a direct, in-situ and instantaneous free-ion activity analysis has been achieved, the principles of metal ion determination will continue to focus upon the distinction of the various species in which metal-ions exist. The use of a 0.45µm filter to operationally define the dissolved metal fraction of a natural water is commonplace, as is the use of electrochemical techniques such as anodic stripping voltammetry, although these methods do not define the free-

ion concentration or activity. Furthermore, discrimination between metal-ligand species is very problematic, whereas some simple inorganic species can be measured or modelled with relative precision, other forms such as organic species can be extremely difficult to isolate and measure. The range of organic ligands is very large with molecular weights in the range of ~100 to 100'000 or greater. Although many organic ligands have been categorised (e.g. amino acids, sugars, uronic acid and carboxylic acids), Pesavento *et al* (2001) recognise that there may be strong ligands of metal ions not yet completely identified.

If a given species is thermodynamically favoured in a given environment, the actual existence of that species may be governed solely by kinetic factors. Moreover, the nature of some species may be altered by external factors such as photo-degradation, particularly species containing organic ligands. Given these limitations, several analysts have sought not to identify individual components, but to categorise metals in terms of their lability fraction, various schemes of this type are examined later in this review.

#### *Studying lability*

Techniques exist, such as ion selective electrodes (ISE), which only measure the free ion concentration (or more accurately activity). Other methods, such as anodic stripping voltammetry (ASV) determine only the very labile (~2ms) fraction, this includes very labile species such as  $\text{Pb}(\text{glycine})^+$ , as well as the free metal ions. These results are often used to state the concentration of the bioavailable metal in solution and can be compared with the total metal concentration to determine the labile fraction. Total metal is generally obtained by acid digestion of the sample followed by an appropriate analytical method, e.g. flame-atomic absorption spectroscopy for more concentrated samples, or inductively coupled plasma mass spectroscopy for trace concentrations. However, these techniques do not allow for the degree of lability to be measured and offer no indication of the form in which the metal is bound, i.e. organic or inorganic.

#### *Use of ion-exchange resins in lability studies*

Many ion-exchange resins have been produced that are made up of ligands bound to polymers. Several of these resins have been used in natural waters research to concentrate metals from solution onto the resin active sites. Table 2 outlines some of the available resins and their functional groups.

Table 2. Resins and their functional groups

Resin	Functional group	Binding sites	References
Chelex-100	Iminodiacetate	Tridentate	Bio-rad, 2004; Wu & Lau, 1996
Poly(4-vinylpyridine)	Pyridine	Monodentate	Wu & Lau, 1996
Amberlite IR-122	Sulphonate	Monodentate	Wu & Lau, 1996
Sephadex SP C-25	Sulphonate	Monodentate	Wu & Lau, 1996
Amberlite CG 50	Carboxylate	Bidentate	Pesavento <i>et al</i> , 1994
Retardion 11A8	Quaternary ammonium group and carboxylate	(Amphoteric)	Samczyński & Dybezyński, 1997

The amount of metal ion sorbed onto the resin will be a function of the concentrations of the competing metals and ligands as well as the contact time of the sample with the resin and the binding capacity of the resin (stability constants of the metal-ligand species). The greater the binding capacity of the resin the greater the capacity of the resin to overcome the stability of certain ligands, i.e. higher binding capacity lead to sorption of not only free metal ions but also those metal ions in less stable complexes than the resin-metal complex. This function can be utilised to study the labilities of metal species in solution. The contact time can also be varied to give different degrees of lability.

Of the resins stated in Table 2, only Amberlite CG50 and Chelex-100 have been used routinely in aquatic research. Amberlite CG50 is a weak cation exchanger and is less efficient than Chelex, which has a functional group with a high affinity for trace metals. This affinity may be a result of the strong binding between nitrogen and the d block metals as well as the stability inferred by further binding to the two adjacent carboxylic groups. Smaller, more abundant ions such as sodium or magnesium tend only to bind to the carboxylic groups, thus making Chelex ideal for the separation of trace metals from bulk solutions. Of the resins studied by Wu and Lau (1996) Chelex was found to be the most effective in taking up the soluble fraction of three metals studied (Cd, Pb and Zn) at low ambient concentrations (0.1-10 mg l<sup>-1</sup>). Use of Chelex is substantial in both speciation studies and for pre-concentration of trace metals before analysis and its uses form the main focus of this review.

### *Aims*

This aim of this review is to investigate the use of the ion-exchange resins, principally Chelex-100, in natural waters research. This will involve investigating resins used in conjunction with diffusive gels, Chelex column and batch techniques, as well as reviewing speciation schemes utilising Chelex.

## Part 2 Properties of Chelex-100

### 2.1 Basic resin properties

Chelex-100 resin (stated from this point as Chelex) is a styrene divinylbenzene copolymer containing iminodiacetate ions which act as chelating groups in binding polyvalent metal ions (Bio-Rad Laboratories, 2004). A study by Loewenschuss and Schmulker (1964) on Dowex A1, an unpurified form of Chelex, concluded that the chelating ligands within the resin were sufficiently spaced that a metal ion is unlikely to bind to more than one iminodiacetic acid (IDA) ligand. However, Atzei *et al* (2001) show that a metal-ion may bind with up to four resin functional groups. Figure 1 shows the basic polymer structure of the resin. The exact nature of the polymer will be dependent upon the fraction of cross-linking molecules and the isomerism of the monomers.

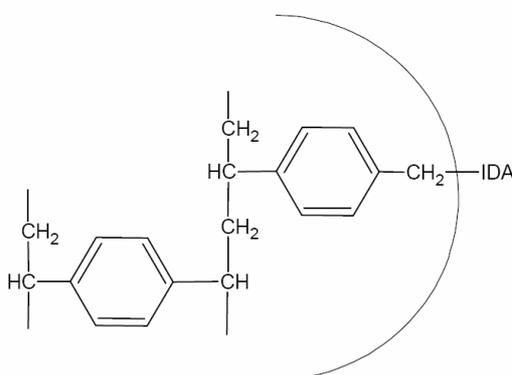


Figure 1. The basic structure of the styrene divinylbenzene copolymer (Nakayamaa *et al*, 2002).

Chelex has a high selectivity for transition metals in contrast to the alkali earth elements making it ideal for separation of trace metals from natural water matrices. Protonation at low pH means that IDA can be considered as triprotic (Napoli, 1972). Figure 2 shows the change in structure of the resin functional group (IDA) with increasing pH.

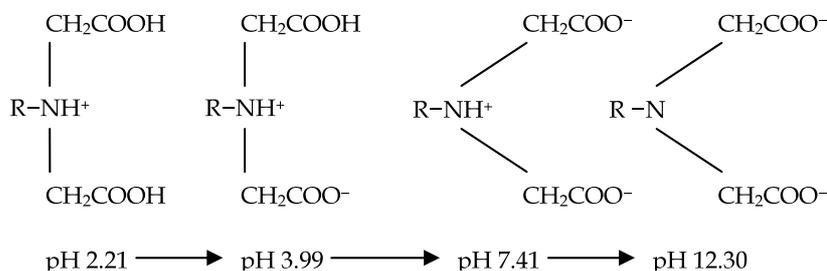


Figure 2. The change in structure of Chelex resins functional groups with increasing pH (Bio-rad, 2004). R represents the polymer structure.

Figure 3 shows a theoretical titration curve for IDA calculated using the Windermere Humic Aqueous Model VI (WHAM6; Tipping, 1998). Equilibrium constants used were from Sillen (1964 & 1971) and Martell & Smith (1974 & 1982).

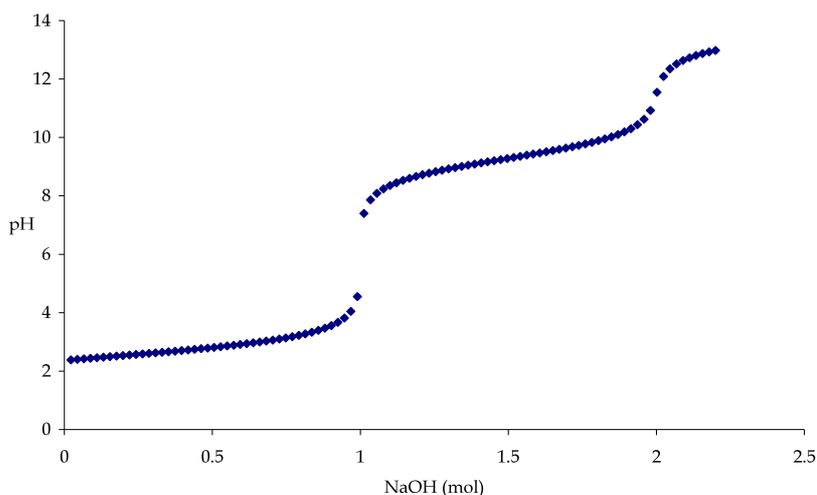


Figure 3. Theoretical titration of free iminodiacetic acid. Titration of 1M IDA with NaOH.

This Figure shows both of the equivalence points that were identified by titration of Dowex A1 by Van Willigen & Schonebaum (1966). However, the first equivalence point at approximately pH 6 is slightly elevated in the Dowex titration and the second equivalence point is better defined in the modelled plot.

#### *Resin forms and mesh sizes*

Chelex is commercially available in two forms, sodium and iron (III). The sodium form is the more widely used in natural waters research, although the iron form has been used for some novel applications. The sodium form can be readily interconverted to other forms such as calcium, ammonium and hydrogen. Use of the sodium and ammonium forms within a column can be problematic as the resin undergoes volume changes when its ionic form is altered, these forms being subject to shrinkage when transfer of multivalent metal ions and  $H^+$  occurs (Figura & McDuffie, 1977). This may be due to the occurrence of ionic cross-linking involving divalent metal ions or the influence of hydrogen bonding when in the  $H^+$  form (Davidson, 2005). These volume changes are allowed to occur due to the low covalent cross-linking in the resin polymer (Atzei *et al*, 2001). This reason for the volume reduction also suggests that binding to more than one ligand occurs. Many analysts use the calcium form to overcome this problem.

The sodium form is available in three mesh (bead) sizes; 50-100, 100-200 and 200-400 mesh (equivalent to a wet bead size of 300-1180, 150-300 & 75-150 $\mu$ m respectively). Bio Rad (2004)

state that the large bead size (50-100 mesh) allows rapid flow rates and therefore the ability to process large volumes of solution. However, use of faster flow rates may reduce resolution by excluding sorption of less labile species. Pakalns *et al* (1978) suggest that the smaller beads (100-200 mesh) can achieve higher resolution but require slower flow rates when compared to the large beads (50-100 mesh). The study also states that the smaller bead size give less efficient recovery of metals from the resin, with 50-100 mesh requiring at least 25 ml of acid and 100-200 mesh requiring more than 100 ml of acid for complete trace metal elution, thus adversely affecting the concentration factors. This observation is contrary to what might be expected if slow diffusion based transport to the inner binding-sites affected the elution efficiency. Furthermore, the small mesh resins (100-200 and 200-400 mesh) are widely used and problems associated with insufficient concentration factors are not reported. Where a technique provides incomplete elution, results can still be interpreted provided the elution efficiency is known and predictable.

The resin has a pore size of approximately 1.5nm consequently excluding large molecules and colloidal particles, thus providing effective separation of ionic metal from colloiddally associated metal species (Batley, 1989). Florence (1977) reports that Chelex has a molecular weight exclusion limit of 500, corresponding to a pore size of approximately 15Å (Bio-Rad report an exclusion limit of 3'500). A check using dye exclusion techniques found that dyes of an estimated size of >25Å were excluded, furthermore, the study also found that hydrated iron oxide colloidal particles are not retained by the resin and little or no adsorption of colloids occurs on the surface of the resin beads as the concentration of binding sites on the bead surface is negligible compared to the total number of binding sites.

#### *Effects of sample pH on resin sorption efficiency*

The efficiency of Chelex sorption (i.e. the fraction of trace metal retention) is partly a function of the pH of the sample. A study by Arroza and Rengan (1999) shows that sorption of Zn and Cd was maximised when the pH was greater than 5. Figura and McDuffie (1977) concluded that retention of several trace metals (Cd, Co, Cu, Ni and Zn) is complete at approximately pH 6.5 and that at lower pH slower resin sorption kinetics can lead to poor trace metal retention (due to incomplete equilibration during the contact time). Many analysts use a buffer in the sample to avoid pH changes during processing, although buffers have also been applied to the Chelex rather than the sample (e.g. Buckley, 1985).

Analysis of trace metals that form anionic complexes with  $\text{OH}^-$ , may require a lower pH for adsorption to occur. At  $\text{pH} > 6.2$  the dominant aluminium species is  $\text{Al}(\text{OH})_4^-$ , this will not be

substantially sorbed by Chelex. Kozuh *et al* (1997) found that adjustment to a pH < 4 is required to achieve near complete sorption of Al<sup>3+</sup> (>98.7%). Pai (1988) found that above the pH thresholds of >5 for Fe and >7 for Cu & Pb, part of these elements may pass through a Chelex column. This is due to the formation of hydroxides which are too large enter the pore structure of the resin. Furthermore, other metals can also complex with simple anions, such as Zn or Hg with chloride (forming ZnCl<sub>4</sub><sup>2-</sup> and HgCl<sub>4</sub><sup>2-</sup> respectively). Therefore, the natural speciation within the sample matrix must also be considered when attempting to concentrate given metals onto the resin.

## 2.2 Stability constants of metal-resin complexes

When an aqueous solution is in contact with the resin, ions will bind to the resin in an order that is largely dependent upon the stability constants of the metal with the resin (Loewenschuss & Schmuckler, 1964). Many stability constants for IDA are documented (Martell & Smith, 1974, 1982; Sillen, 1964, 1971); however, the application of these constants to the Chelex IDA group may not be ideal. Pesavento *et al* (1993) found that the selectivity of some divalent metal ions is poorer than expected from the complexation by IDA in aqueous solution. Results for calcium, zinc and cadmium were lower than predicted, although copper and nickel gave results in good agreement. The study concluded that chelation by the IDA prevails if strong complexes are formed and complexation by the carboxylate groups prevails when metal ions with lower complexation constants with the IDA are involved. As complexation within the resin can be completed in one step (Kaneko & Tsuchida, 1981) the complexes of the resin may have a greater stability than those formed with IDA in solution, as free IDA complexation may involve more than one step or more than one IDA molecule (Van Willigen & Schonebaum, 1966). Schmuckler (1965) suggests that the bond strength of a resin-metal may be an order of magnitude stronger than the equivalent bond with a free chelating ligand.

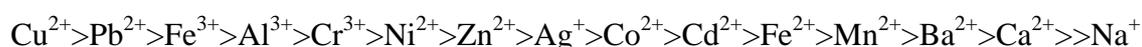
In the majority of Chelex uses, the stability constants of metal-resin complexes are not required providing that the resin binding sites are in sufficient excess to the metals that will be sorbed, i.e. for preconcentration of trace metals from seawater. However, a recent study by Senn *et al* (2004) has focused on the use of an IDA resin (Toyopearl AF-Chelate 650M) to enable calculation of copper concentrations by allowing the resin chelating sites to equilibrate with surrounding water (the full technique is reviewed in section 5). As no stability constant data exists for the selected resin, IDA constants are used as an approximation of the resin-IDA values. Limited studies have been undertaken on Dowex A1 (Van Willigen & Schonebaum, 1966; Loewenschuss & Schmulker, 1964) and Chelex (Sunda, 1984) to determine the stability constants of several metal-resin complexes. However, given this recent work and the potential usefulness of these resins in

equilibrium based studies, the determination of critical stability constants for Chelex and other ion-exchange resins would be a beneficial development.

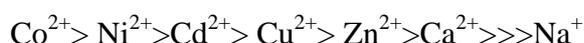
#### *Distribution coefficients and the selectivity of Chelex*

The distribution coefficients of metals between the resin and solution phase within a given environment are simpler to obtain than stability constants. Bio-Rad (2004) have classified the selectivity factors of the resin using  $\text{Zn}^{2+}$  as the reference cation.

- The approximate series in nitrate solution



- The series at pH 9 in presence of 1.5M  $(\text{NH}_4)_2\text{SO}_4$



Bio-Rad (2004), report that  $\text{Hg}^{2+}$  is high in the selectivity series in nitrate media, but low in the presence of chloride with which it forms a complex.

#### *Estimating stability constants*

Where few critical stability constants exist, they may be estimated by comparison with a similar metal-ligand complex for which more data exist. Correlations can exist between logarithms of equilibrium constants of related sets of compounds as  $\text{Log } K$  is proportional to the free energy ( $\Delta G^\circ$ ). These relationships are termed linear free-energy relationships (LFERs; Brezonik, 1990) and are linear because replacing one functional group for another does not alter the transition state of the metal-ligand reaction in an important way, only its energy. Small changes in structure that alter the free energy of the reactants relative to products cause proportional changes in the energy of the transition state. When structural changes become large enough the structure and form of the reaction transition state is altered and the energy change is not accounted for by LFERs (Stone & Morgan, 1990).

Figure 4 shows comparisons of three compounds with IDA. Figure 4a demonstrates that the selection of an inappropriate 'partner-ligand' significantly reduces the accuracy of any estimated values. Comparison with amine containing compounds gives a more acceptable data fit. Figure 4b shows how the constants can be estimated graphically, although the equation of the best-fit line can also be used to calculate the value.

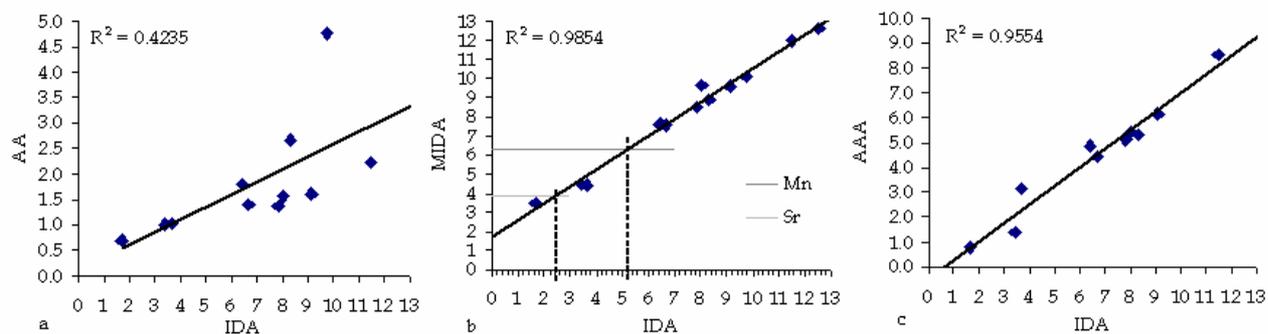


Figure 4. Comparison of metal and proton critical stability constants of IDA with three other ligands. AA= acetic acid, MIDA= methyliminodiacetic acid and AAA= aminoacetic acid. Both axes of each graph represents log stability constants at zero ionic strength and 25°C. See text for discussion.

Log values of Mn and Sr estimated from Figures 4b & c are accurate to log 0.5 and log 0.7 respectively. Although when absolute values are considered the errors can be expressed as approximately 500% and 300% respectively. Thus, demonstrating the caution that is required when using these estimates. For further examples on the use of LFERs see Martell & Hancock (1996).

### Part 3 Chelex column methods

The most widespread use of Chelex is its use within a column, principally for preconcentration of metals, but also for lability studies. In both preconcentration and lability studies the resin is used as an infinite sink for the metals of interest. The approaches taken to these types of study are very similar with the major difference being that samples for lability studies should not be pre-treated (e.g. buffered) as this affects the chemistry of the sample (sample pre-treatment methods for preconcentration studies are outline in appendix 1). Column preconcentration is carried out to determine the total metal concentration, lability studies seek to determine the metal that is associated with species that can undergo Chelex sorption within a given period of time (see section 5 for examples of lability studies).

There appears to be no consistent approach to Chelex column use between analysts, with wide variations in flow rates, sample volume, resin mass & form (including column dimensions) and pre-treatment of samples. There are also large variations in the recovery rates reported. Table 3 gives selected parameters used by several analysts and shows the wide variability between studies.

Table 3. Selected parameters in several Chelex column studies (both preconcentration and lability studies).

Study	Resin mass (g) <sup>1</sup>	Sample volume (l)	Elution volume (ml)	Flow rate (ml min <sup>-1</sup> )	Concentration factor <sup>2</sup>
Kingston <i>et al</i> , 1978	3.8	1	10	1	100
Pai <i>et al</i> , 1990	2	1.28	5	4-5	~250
Rasmussen, 1981	n/r	~1	10	1	~96
Figura & McDuffie, 1979	1.3	1	25	2-3	40
Bruland <i>et al</i> , 1979	n/r	4	n/r	3.5-4.5	120
Vazquez <i>et al</i> , 1996	1.3	0.25	n/r	2.0±0.2	n/r
Vermeiren <i>et al</i> , 1990	1 & 3	0.75	25	2.5	30

n/r = values not reported. <sup>1</sup>Reported as mass of initial Na form. <sup>2</sup>Approximate values estimated from sample and eluent values quoted, otherwise values are as reported. Concentration factors do not account for any additional treatment which may be undertaken to further the concentration factors e.g. evaporation.

The basic method used in a frequently replicated study is outlined below; this is followed by a discussion of the individual parameters. For information on the apparatus used refer to the references in Table 3.

Many analysts use a method similar to that developed by Kingston *et al* (1978) and has been applied to both freshwaters and seawaters. Approximately 4g of resin (~6 ml) were placed into a tapered column. The resin was washed with four 5 ml aliquots of 2.5M nitric acid to elute any trace metal contamination in the resin. This was followed by rinsing with two 5 ml volumes of water to remove excess acid, the resin was then converted to the ammonium form. A 1 litre buffered sample was allowed to flow through the column, initially at a flow rate of 0.2 ml min<sup>-1</sup> to

allow for shrinkage of the resin (approximately halves in volume and takes 2-3 minutes), the flow rate then being increased to  $1 \text{ ml min}^{-1}$  (~17 hours for entire sample to pass through column). A pH of 5-5.5 was chosen as the efficiency for chelation of Co and Cu has been shown to decrease above pH 6 (Kingston *et al*, 1978; and references cited therein). Although, this may be due to effects of the seawater matrix as Figura & McDuffie (1977) found that retention of these metals was complete (>99.9%) at ~pH 6.5 in a synthetic river water. (Kingston *et al* (1978) buffered the sample by adjusting the pH to 5-5.5 with drop-wise addition of  $\text{NH}_4\text{OH}$  followed by addition of 5ml of ammonium acetate (per 1l of sample)).

### *Resin Preparation*

A resin rinsing step similar to the one outlined above is undertaken on all column studies, it is mostly undertaken in situ in the column but occasionally as a batch before column setup. This is carried out principally to remove trace metals present on the resin. However, Pakalns *et al* (1978) highlight the importance of this step to also remove  $\text{OH}^-$  that is present in the pore structure of the resin in its supplied form, thus avoiding precipitation of metal hydroxides that may block the column.

The form of the resin is significant, as shrinkage of the sodium and ammonium forms may lead to channelling in the column. Where these forms are used, slow initial flow rates generally overcome the problem of channelling. However, in lability studies, the contact time of the sample with the resin is an important factor and adjusting flow rates may affect results. Problems can also occur when using the hydrogen form. Florence & Batley (1977) found that when using the  $\text{H}^+$  form very little metal was retained until approximately 1l of a seawater sample was passed through the column. Immediate retention was achieved with the  $\text{NH}_4^+$  and  $\text{Na}^+$  forms. The  $\text{H}^+$  form may also affect the pH of the effluent, which may disturb the equilibrium of the remaining species, this is an important consideration in lability studies as the effluent may be further studied.

The calcium form has been used in some studies and has several benefits, the pH of the sample and column effluent are not substantially changed, thereby not affecting equilibrium. As the Ca form does not undergo shrinkage a steady flow can be maintained without channelling in the column (Figura & McDuffie, 1977), making it ideal for lability studies as consistent flow rates can be applied. As calcium is low in the Chelex selectivity series there is little competition for binding sites between Ca and most trace metals.

Baffi & Cardinale (1990) sought to use purified seawater (seawater passed through a Chelex column to strip trace metals) to convert the resin into a 'sea-form', however, high blank values were found for Cu & Fe, possibly due to ineffective removal of the trace metals from the seawater sample. If this technique were required, synthetic seawater made up from high purity salts would be more desirable.

#### *Column size/resin quantity*

The most fundamental factor in deciding the resin quantity is ensuring that the concentration of binding sites is in excess compared to the concentration of trace metals that are to be exchanged. One gram of dry resin has two milli-equivalents of binding sites. Assuming that the concentrations of trace metals in the studies in Table 3 do not grossly exceed those reported for seawater in Riley & Taylor (1972), then there is a significant excess of resin binding sites for all the studies. The large excess of binding sites suggests that competition effects will not adversely affect the trace metal retention.

The dimensions of the column are not a dominant factor providing the contact time of the eluent with the resin is sufficient and that channelling is avoided. Solzberg & Rosin (1977) found that when using a peristaltic pump channelling occurred with small (3 or 6 mm) internal diameter (i.d.) columns, whereas this was not evident in a 10mm i.d. column. A typical column is approximately  $0.8-1.0 \times 10^{-12}$  cm, however, use of resin forms that undergo shrinkage may lead to the height of the resin in the column reducing by as much as ~50%. In lability studies the resin quantity, or more accurately the void volume of the resin, coupled with the flow rate, will determine the contact time of the water sample with the resin (void volume also takes account of the bead size).

#### *Flow rates*

The flow rates used need to be a balance between the sorption efficiency required and the time involved in processing an entire sample. Paulson (1986) showed that decreasing the flow rate to  $0.2 \text{ ml min}^{-1}$  is effective in increasing retention. However, a one litre sample would take nearly  $3\frac{1}{2}$  days to process. Flow rates more commonly used vary from  $1-4 \text{ ml min}^{-1}$ . Florence & Batley (1976) varied flow rates between  $0.5$  and  $3 \text{ ml min}^{-1}$  and found no effect on trace metal retention. Conversely, Pai (1988) found that reducing flow rate from  $4$  to  $1 \text{ ml min}^{-1}$  was equivalent to doubling the column size in terms of trace metal retention. However, Pai also concluded that a column containing 2g of resin and a flow rate of  $4 \text{ ml min}^{-1}$  provided >98% retention efficiency for Cd, Co, Cr, Mn, Ni, Pb and Zn in a buffered seawater sample.

In laboratory studies the term 'contact time' is used to describe the effects of both flow rates and column size (i.e. resin quantity). The contact time ( $t_c$ ) being defined as the average time of the sample contact with the resin phase (DeMora & Harrison, 1983).

$$t_c \text{ (min)} = \frac{\text{void volume of column (ml)}}{\text{flow rate (ml min}^{-1}\text{)}}$$

Void volumes (the space occupied within the beads and between bead pore spaces) are difficult to quantify, nevertheless some studies have estimated values of  $t_c$ . Figura & McDuffie (1979) estimate  $t_c \approx 6\text{-}9\text{s}$  for 1.3g of resin and  $2\text{-}3 \text{ ml min}^{-1}$  flow rate, Pai (1988) estimated  $t_c \approx 7.5\text{s}$  for a void volume of 0.5ml and a flow rate of  $4 \text{ ml min}^{-1}$ .

#### *Pre-elution rinsing step*

An important step when pre-concentrating trace metals from seawaters or hard waters is the removal of 'bulk ions' (e.g. Na, Ca, Mg & Cl) that may cause interfere with the final analysis technique (for example Ca and Mg severely suppress some analytes in graphite furnace atomic adsorption spectroscopy (Kingston *et al*, 1978)). This rinse is typically done by passing approximately 10-20 ml of 1M ammonium acetate, per gram of resin, through the column, followed by a rinse with 10ml of water.

Greenberg & Kingston (1983) observed a reduction in concentrations by a factor of  $\geq 10^5$  for chloride,  $\geq 10^7$  for sodium and  $\geq 10^3$  for bromide after the preconcentration and rinsing steps. Thus demonstrating the efficiency of the resin in separating trace metals from the seawater matrix. However, the rinsing step was found to remove a small percentage of some trace metals from the column (5.5% for Th and  $\sim 0.1\text{-}1\%$  for Sn, Sc, Mo, Fe & Cr).

#### *Elution of bound metals*

The elution step is carried out in most studies with small quantities of 2M nitric acid ( $\text{HNO}_3$ ), with many studies performing a stepwise elution with 1-5ml aliquots. Pai *et al* (1990) suggests that bulk volume elution (e.g. one 25ml treatment) may be inefficient as vertical convection occurs in the top layer of the column, leading to long tailings of the eluate. The study concluded that approximately 7ml of  $\text{HNO}_3$  was all that was required to elute all metals when using 1ml stepwise aliquots. Furthermore, the first 2ml could be discarded as they were shown to contain no trace metals.

A number of studies have observed that not all trace metals can be eluted effectively with HNO<sub>3</sub>. Pakalns *et al* (1978) found that elution by 2M hydrochloric acid (HCl) aided removal of manganese from the column and recommend elution with HCl in addition to HNO<sub>3</sub>. A comprehensive investigation into elution efficiency by Riley & Taylor (1968) concluded that HNO<sub>3</sub> was the most useful reagent as it eluted many metals. However, several metals were found to require other eluents as outlined in Table 4.

Table 4. Preferred reagents for elution of selected metals (Riley & Taylor, 1968)

Metal	Eluent
Bismuth	2M Perchloric acid (HClO <sub>4</sub> )
Thorium	2M Sulphuric acid (H <sub>2</sub> SO <sub>4</sub> )
Cobalt	2M Hydrochloric acid (HCl)
Vanadium (V)	4M Ammonium hydroxide (NH <sub>4</sub> OH)
Molybdenum (VI)	4M Ammonium hydroxide (NH <sub>4</sub> OH)

There is no reason why one strong acid should be favoured over another, as the competitive effect of two strong acids at the same strength should be equal. The effect could possibly be the result of interference in the analytical method by the acid anion.

## **Part 4        Speciation studies utilising Chelex**

### **4.1        Chelex batch methods**

Chelex batch methods employ resin preparation, sample/resin quantities and elution techniques similar or identical to those utilised in column studies. However, batch techniques are seldom used for preconcentration with the most common use being in speciation (lability) studies, which may include analysis of column effluent and/or untreated samples (which may or may not be filtered).

Batch techniques involve increasing the contact time of sample with resin by stirring or shaking a mixture of the two in a closed vessel for a predetermined time. It has certain advantages over column techniques, namely that the preconcentration factors that can be obtained may be larger due to Chelex sorption of slower lability species and secondly, that it enables calculation of the inert fraction by analysis of the bulk solution following batch equilibration.

#### *Equilibration time*

The time allowed for batch-sample equilibration must be sufficient to allow a significant quantity of the slowly labile fraction to sorb to the resin. Figura & McDuffie (1980) recognise that in this method, where most of the sample is not exposed to a local excess of resin, the uptake of the metal will be limited not only by dissociation kinetics of the metal-ligand complex (as in the column method), but also by the finite rate at which the free metal is brought into the locality of the resin phase. Thus, the effectiveness of the technique will also depend upon the method of agitation used.

Chelex batch equilibration timescales used in recent studies range from 16 to 168 hours. Time series analysis of Chelex batch experiments by Lu *et al* showed that uptake of selected trace metals was initially rapid and with the exception of Cu, was maximised within approximately 35 minutes (Pb, Cu & Cd studied). Hart & Davies (1977) found no discernable difference in the concentrations of samples equilibrated for 16 and 168 hours. However, Chakrabarti *et al* (1993) observed that copper in a snow sample continued to be subject to Chelex sorption after 72 hours. This highlights the variability in the strengths of the ligands found in natural water samples. Several analysts (Figura & McDuffie, 1980; Vazquez *et al*, 1996) use a standard time of 72 hours. However, given the above studies it is recommended that at least two time points should be obtained to check that the equilibration time chosen is adequate.

### Part 4.2 Speciation schemes

Several speciation schemes exist where Chelex batch and column techniques are coupled with other analytical and/or separation techniques to operationally classify the lability of species, rather than identifying actual species. The binding spectrum in Figure 5 (Figura & McDuffie, 1979) shows a simple scheme involving separation of four operational fractions. These principles form the basis of many speciation schemes utilising Chelex, with some schemes expanding the categorisations with other supplementary techniques. Three schemes will be reviewed in more detail in order of their experimental complexity.

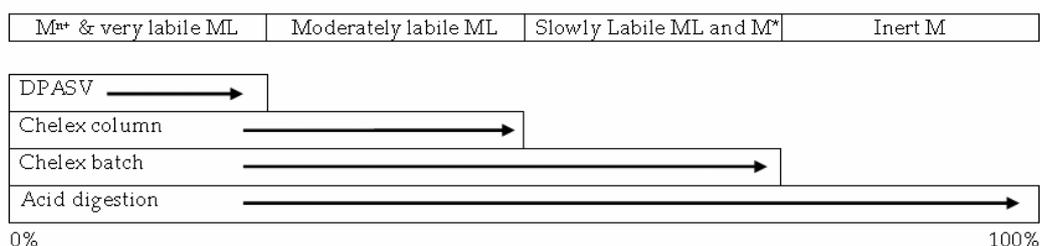


Figure 5. The binding spectrum for soluble trace metal species (Figura & McDuffie, 1979). ML represents a metal-ligand complex,  $M^*$  represents metal bound to colloidal particles and DPASV= differential pulse anodic stripping voltammetry.

#### Figura and McDuffie (1980) scheme

This scheme differentiates trace metal species by their relative lability and utilises both column and batch Chelex methods coupled with (differential pulse) ASV analysis. Total metal is determined by acid digestion and subsequent ASV analysis. A 'very labile' fraction is ascertained by direct ASV of a filtered sample with an added buffer. 'Moderately labile' results are obtained from passing the sample through a Chelex column with the ASV 'very labile' fraction subtracted from the result. The 'slowly labile' fraction is classified as the trace metal species in the column effluent that is equilibrated with a Chelex batch for 72 hours. The solution from the equilibrated batch is then acid digested to enable determination of the 'inert' fraction. The inert fraction is defined in the method as 'extremely stable metal-ligand complexes, non-labile complexes or metal strongly adsorbed to colloidal matter' (Figura and McDuffie, 1980). Figure 6 shows the flow chart for this speciation scheme.

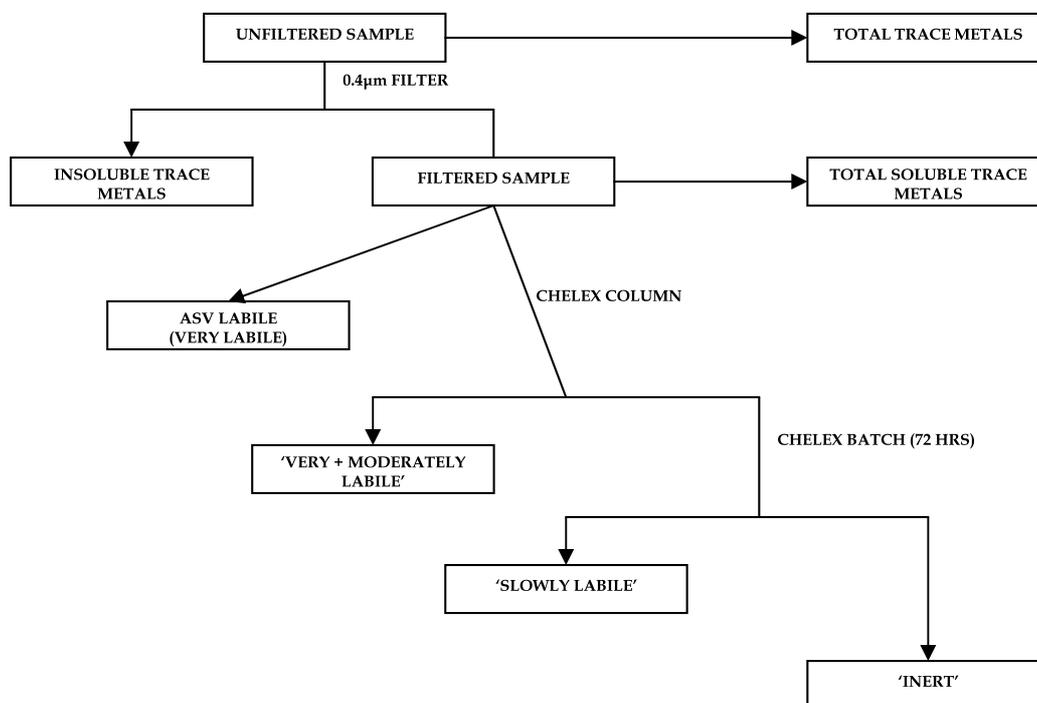


Figure 6. Flowchart of the Figura & McDuffie (1980) trace metal speciation scheme.

Batley (1983) suggests that this scheme does not provide mutually exclusive separations, in that the ASV determined 'very labile' fraction will not necessarily be wholly retained by the Chelex column. However, in a prior study by Figura & McDuffie (1979) they suggest that the column technique enables pre-concentration of the entire labile fraction. This uncertainty could be resolved by analysis of the column effluent with a suitably sensitive technique. Further uncertainty may exist where the use of too negative a plating potential ( $E_p$ ) in ASV analysis may lead to the direct reduction of complexes that are considered non-labile to this method (for example Cu-EDTA); therefore the selection of  $E_p$  should be considered carefully (Figura & McDuffie, 1979; 1980).

#### *Batley & Florence (1976) Scheme*

This scheme seeks to further classify the groups of complexes by distinction of the organic and inorganic fractions in all categories. Total metal is measured in a similar way with boiling of an acidified sample. However, the soluble fraction (defined as that which passes through a  $0.45\mu\text{m}$  filter) is subjected to direct ASV analysis or acid digestion then ASV analysis. These determinations are undertaken on an untreated sample (filtered only), after passage through a Chelex column at a flow rate of  $1\text{ml min}^{-1}$ , after UV treatment of the sample, and after UV treatment and passage through a Chelex column. The UV treatment involves addition of  $0.05\text{ml}$  of 30%  $\text{H}_2\text{O}_2$  per  $100\text{ml}$  of sample followed by exposure to a  $550\text{W}$  UV lamp for a six hour period.

The full classification scheme obtained is shown in Figure 7, seven groups of species are defined based upon four treatments of both the ASV labile sample and an acid digested sample.

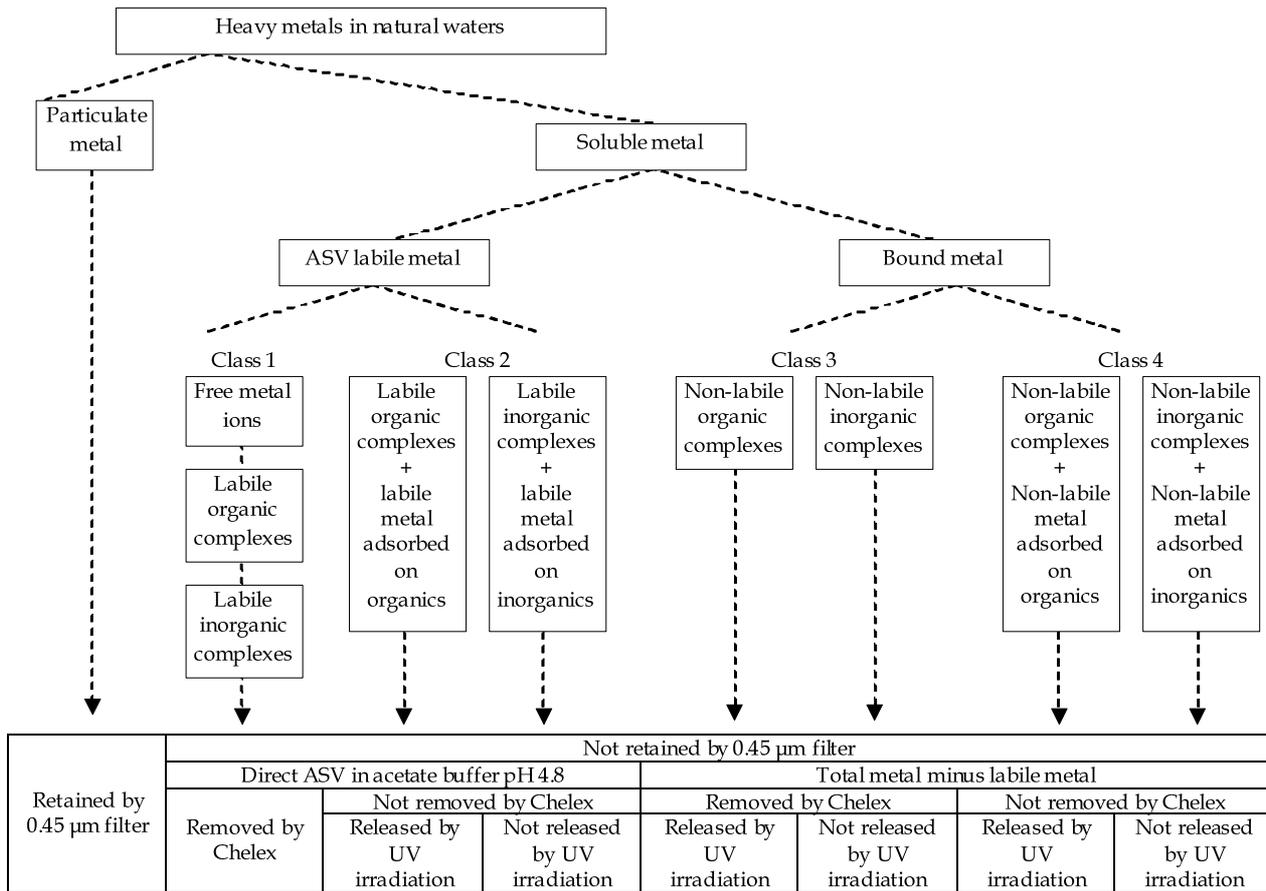


Figure 7. Flow chart for the Batley & Florence (1976) speciation scheme

Given the strength of the Chelex-metal complexes and the ability of the resin to compete with all but the strongest ligands in solution, class 2 species (as defined in Figure 6) would be more accurately defined as moderately labile or Chelex-column non-labile. Moreover, given the order of magnitude difference in equilibration time, significant ASV labile metal should not pass through a Chelex-column unless too negative a stripping voltage was used (as discussed above). However, the authors do define labile as, those species which are reducible at the selected deposition potential. It is therefore assumed that a deposition voltage is chosen that will strip metals from all but non-labile species, in contrast to Figura & McDuffie (1980) who selected a voltage to strip only free metal ions and metals from very labile complexes. It is also possible that the class 2 adsorbed labile metals are associated with colloids which do not have sufficient contact with iminodiacetate sites, due to size exclusion from the resin pores, thereby reducing the Chelex uptake of metals associated with these species.

Batley (1983) quotes an eight hour analysis period for this scheme and suggest that because of this it can not be applied to routine analysis. However, as it does not involve Chelex-batch techniques, full results can be obtained more quickly than the application of other schemes that use batch equilibration times of 16 hours to several days. The potential for overlap within the categories is also recognised. Given that the degrees of lability of the species are not examined and that the labile fraction is assumed to be the ASV labile fraction, overlap will occur. Batley & Florence (1976) state that the possible errors associated with each calculated species concentration may be as high as  $\pm 10\text{-}15\%$ . This scheme does have the advantage that little sample manipulation is required, thus largely avoiding contamination issues.

#### *Chakrabarti et al (1993) scheme*

This scheme is essentially based upon the Figura & McDuffie scheme but further classifies each category into size fractions using ultrafiltration techniques. The molecular weight (MW) cut-offs are, 100000, 50000, 30000, 10000, 5000, 1000, and 500. Although, the authors do recognise that ultrafiltration differentiates metal species by size, shape and charge characteristics rather than by MW. This scheme provides a more comprehensive insight into the physical and chemical characteristics than each of the separate analysis techniques can achieve alone (Chakrabarti *et al*, 1993) as the binding characteristics of a range of ligands with varying sizes can be assessed. This scheme may be very useful as more determinations are undertaken on the types of ligands in natural waters. Better characterisation of ligands will lead to greater predictability of the ligand types within the various size fractions. The scheme is outlined in Figure 8.

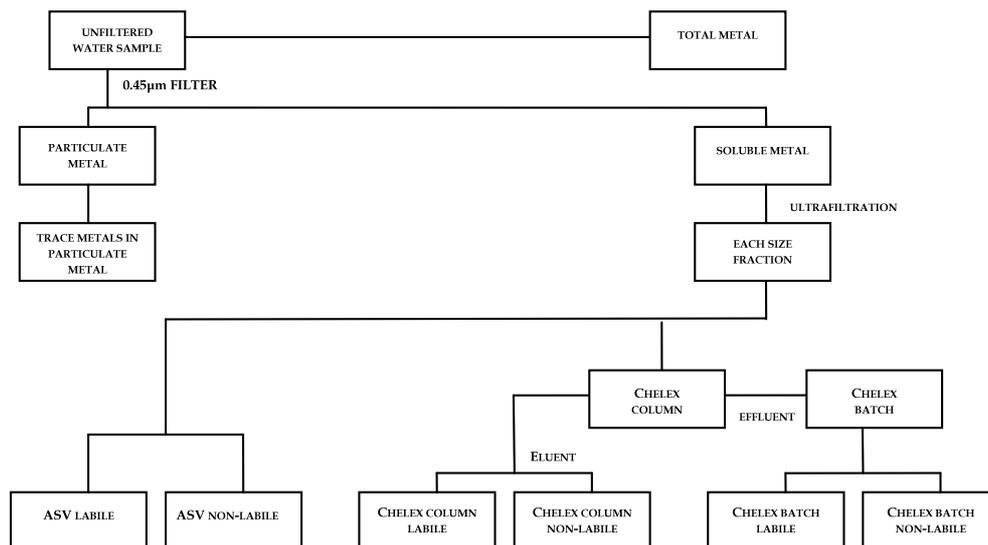


Figure 8. Modified diagram of the Chakrabarti *et al* (1993) speciation scheme.

### *Other schemes*

Hart & Davies (1981) utilise Chelex batch techniques and a dialysis step to enable determination of labile metal in MW complexes of <1000. Furthermore, there are few categories defined, thus providing little insight into the trace metal complexation within the sample. However, it does offer a simple speciation scheme with little sample manipulation and provides a suitable scheme for routine analysis. Florence (1989) suggests a scheme that, as well as Chelex extractions, also includes extraction of lipid soluble metal by extraction with 5ml of 20% *n*-butanol in hexane. This allows the potentially highly toxic lipid soluble fraction to be determined and should be undertaken where bio-toxicity is being considered. Although not suggested as a speciation scheme, Pesavento *et al* (2001) demonstrate that use of two chelating resins with different binding strengths may give data on the relative labilities of trace metal complexes. Although there will be overlap within the results, this could potentially be used as a supplement to Chelex batch and column methods to further operationally classify labilities of complexes.

### *Applications of speciation schemes*

Speciation schemes offer evaluation of the nature of metal speciation in a natural water. However, the complexity and therefore operational difficulty, of many of these schemes means that they are seldom used for studying speciation, with many analysts seeking to exclusively define the labile fraction and to use these data in considering the bioavailability. Nevertheless, it may be the case that these schemes could be an aid to defining the fate of inorganic pollutants in the environment. The categorisation of slowly-labile and non-labile complexes may aid understanding in the long-term fate of metal contaminants. The determination of the size fractions of these metal complexes may also help in identifying the ligands responsible for long-term binding. However, the overriding interest in applying trace metal studies to bioavailability has meant that little work has been undertaken on assessing the behaviour and fate of less labile ligands. These speciation schemes demonstrate that the whole binding-spectrum can be investigated (to varying degrees of complexity) and providing there are put into a relevant context they can provide useful data.

## Part 5 Recent developments

Recent developments in trace metal research have utilised diffusive gels in conjunction with IDA resins. Diffusive gradients in thin-films (DGT; Davison & Zhang, 1994) has been developed over the past decade and uses Chelex as an infinite sink for trace metals diffusing through a fixed layer of gel. A recent study has suggested an equilibrium based sampler (EBS; Senn *et al*, 2004) which utilises an IDA resin that is allowed to equilibrate with natural waters, which enables back calculation of metal concentrations. Both techniques allow in situ analysis.

### 5.1 Diffusion gradients in thin films

DGT utilises a layer of polyacrylamide gel (often termed hydrogels due to their high water content) backed by a further thin layer of gel containing Chelex beads (the smallest available, 75-150 $\mu\text{m}$ ), in close packed form (Davison & Zhang, 1994). Transport within the diffusive gel is restricted to diffusion and thus transport to the resin is a function of the gel thickness. Figure 9 shows a schematic of the bulk ion concentration in a gel assembly (bulk ions can be defined as free metal ions plus those ions that may be associated with ligands that will pass through the gel and are Chelex-labile).

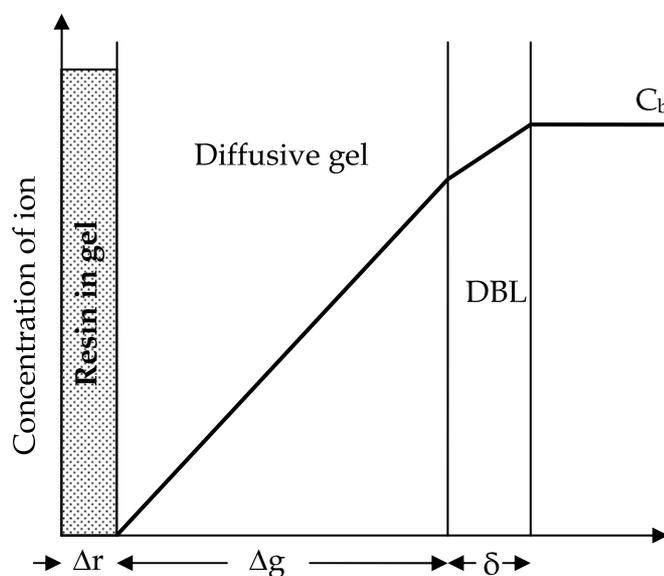


Figure 9. Representation of the free concentration of ionic species in a DGT assembly in contact with a natural water, where the free metal-ion concentration is  $C_b$ . DBL = diffusive boundary layer. (Davison & Zhang, 1994).

The concentration of metal within the resin-gel layer (thickness  $\Delta r$ ) is assumed to be zero as the metals are preferentially bound to the unsaturated Chelex. Transport from the bulk solution to the gel (thickness,  $\Delta g$ ) is assumed to be across a diffusive boundary layer (DBL) of thickness  $\delta$ , where transport is only by molecular diffusion. Ions must first diffuse across this DBL before diffusing into the gel. The gel is reported to have an effective pore size of 2-5 nm thus preventing passage of large molecules. A 0.45  $\mu\text{m}$  cellulose nitrate filter prevents particles adhering to the gel surface.

The flux of the metal to the resin can be calculated using Fick's laws, providing the sampling time, surface area of the sampling device and the thickness of the diffusive gel layer are defined (Davison & Zhang, 1994). Metals are extracted from the resin-gel layer with 1ml of 2M HNO<sub>3</sub>.

What the DGT actually measures depends upon the pore size of the diffusive gel and the lability of metal complexes in solution. All labile inorganic species, free metal ions and labile organic complexes capable of diffusing in to the hydrogel will sorb to the resin sink (Zhang, 2004). The deployment time may have an effect if slowly labile species are present. The concentration of metal bound to Chelex is a function of the deployment time of the device. It is suggested that the capacity of the resin may allow the apparatus to be maintained in situ for up to three months, enabling its use in presenting a record of long-term integrated metal concentrations. The limitations that may exist due to biofouling of the apparatus during extended deployments are recognised (Davison & Zhang, 1994).

As DGT defines metal concentrations using the physical properties of the diffusive gel and surrounding media, and with the resins exclusive role as providing a sink for the metal of interest, a full review of all DGT applications is beyond the scope of this study. However, there are some applications relevant to this review and these are summarised below.

#### *DGT deployment in sediments and soils*

Figure 9 represents the bulk ion concentration in a DGT assembly deployed in a natural water and assumes the bulk solution is well mixed i.e.  $C_b$  is constant. However, this representation is not applicable to deployment in soils and sediments as there may not a constant concentration in the bulk solution. There is diffusion from within the sample and localised desorption from the mineral/organic matrix (i.e. an induced flux; Harper *et al*, 2000). A gradient in metal concentration is established between the diffusive-gel and the sediment/soil bulk solution, the gradient and the distance from the gel where this induced flux has an effect, is dependent upon the deployment time, the response-time and the capacity of the media i.e. solution concentration. These techniques have been developed to allow the measurement of vertical profiles within sediments (Fones *et al*, 2001).

#### *Use of DGT in metal speciation studies*

The potential for gels of varying porosity to discriminate organic and inorganically complexed metals was first suggested by Zhang & Davison (1999). Recent studies have focussed on developing DGT for in situ speciation measurements. By using devices containing hydrogels of

two pore sizes, complexes of different sizes can be discriminated. Inorganic species diffuse through all gels, but larger organic complexes diffuse less freely through more restrictive gels (Zhang, 2004).

The range of categories defined by DGT speciation studies is few compared to those of speciation schemes such as that reported by Florence & Batley. The use of two hydrogels has been shown to distinguish total free ion plus labile inorganic and organically labile complexes (Zhang, 2004), thus providing useful data on the fraction of a metal bound reversibly in organic complexes. The free metal ion can also be numerically estimated from the inorganic plus free-ion data (see Meylan *et al*, 2004 and references therein for examples).

Another approach to using DGT in speciation studies is under development and involves the use of several different binding phases. It is suggested that by designing binding phases that compete with natural water ligands to varying extents, it may be possible to use several different DGT devices to measure metal speciation in natural waters (Li *et al*, 2005). This presents the possibility that a far more experimentally simple yet comprehensive speciation scheme can be developed, which may take the place of the more complex and time consuming schemes reviewed in section 4.

## 5.2 *Equilibrium based sampler*

A recent article by Senn *et al* (2004) proposed a novel method of determining  $\text{Cu}^{2+}$  concentrations in aquatic systems. It differs from DGT in that the diffusive-gel is omitted and the resin-gel is allowed to equilibrate with the sample. As in DGT, a polyacrylamide gel is used to act as a holding reservoir for the resin. A different resin (Toyopearl AF-Chelate 650M) is used in place of Chelex, which has a mean bead size of 65  $\mu\text{m}$  and considerably less iminodiacetate binding sites than Chelex. The reduced number of binding sites per volume of resin allows closer control to be maintained on the number of chelating ligand sites within the device. Furthermore, the larger pore size (and therefore molecular weight exclusion limit) will reduce the diffusion time into the inner resin binding sites in comparison with Chelex.

Preliminary laboratory experiments showed that the equilibrium based sampler (EBS), referred to as 'gellyfish' by the authors, may take at least 12 days to equilibrate, this may restrict the use of the sampler in rapidly changing aquatic systems. However, Senn *et al* (2004) suggest that decreasing the thickness of the resin-gel layer by a factor of four may reduce the equilibration time by a factor of 16. The sampler was not deployed in a natural water in this preliminary study, but was shown to be effective across a range of salinities and copper concentrations in the laboratory.

Elution of the copper from the resin was carried out with 5 ml of 10% HNO<sub>3</sub> left for at least two days. Copper recovery from the resin is reported to be greater than 95%. DGT elution involves smaller volumes and elution times, suggesting the volumes of acid used and the elution time of the EBS may be reduced without adversely affecting the results.

#### *Limitations of the EBS*

The EBS requires knowledge of stability constants to enable the bulk solution concentration to be calculated from the resin eluent concentration. As no data exist for the resin, values are assumed to be the same as the free-IDA constants. The acid is considered as diprotic in the model, i.e. the triprotic nature of IDA and IDA-resins is overlooked. Furthermore, only four metal stability constants are used in calculating predicted copper binding. It is suggested however, that due to the resins large affinity for Cu, the inclusion of other constants (e.g. Zn<sup>2+</sup>) will alter the EBS Cu concentration by less than 5% (Senn *et al*, 2004).

This technique does provide a potentially useful method of measuring Cu concentrations providing the limitations are overcome or recognised. This method may also be deployed to determine the concentrations of a range of metals provided the stability constants are available and that the eluted concentration is sufficient for the analytical technique used. Senn *et al* (2004) suggest that the volume of beads can be increased up to an equivalent of 900µeq/l IDA within the sampler, without compromising the structure of the gel. An increase in the number of binding sites may be necessary to determine less competitive metals or to measure competitive metals in pristine environments, although the equilibration time may have to be increased as a result.

## Part 6 Summary and proposal for further work

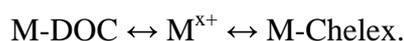
The use of ion-exchange resins, particularly Chelex, is widespread and many procedures for its use are well defined and developed. The concentration factors achieved often allow for simpler and cheaper analytical methods to be used, or metals that may normally be below detection, to be analysed. The range of metals that can be studied using these techniques is vast and providing the optimum conditions are known, good repeatable recovery rates can be achieved.

The application of speciation schemes to the study of trace metal complexation has been less widely undertaken. The complex nature of some of these schemes and the time consuming analysis required has limited the application of these methods. The simpler schemes, despite providing less comprehensive data, can be applied with less time and equipment with useful results. The development of DGT based speciation studies may allow inexpensive, routine analysis to distinguish between organically complexed metals and inorganic/free metals.

Chelex use is currently limited to preconcentration studies, speciation schemes and DGT. In all of these applications it is used as an infinite sink, where kinetic effects may be a factor (i.e. in column analysis) but competition effects are not significant as the binding sites are in excess compared to the available competitive cations. However, the next phase in the evolution of Chelex/IDA-resin use may be in equilibrium-based studies such as the equilibrium-based sampler. These techniques may ultimately allow calculation of the free-ion concentrations of trace metals in natural waters. Nevertheless, the determination of stability constants for metal-Chelex species will be required as free-IDA constants are not comparable to resin-IDA constants.

### *Project proposal*

If Chelex (without diffusive gel) is allowed to directly equilibrate in-situ with a natural water, this will allow a given concentration to be eluted from the resin. If a relationship can be established between the environmental concentration and the eluate concentration and considering other factors including (but not limited to) pH, ionic strength and temperature, the free ion concentration may be calculated from the Chelex sorbed concentration. This technique may allow  $\text{pg l}^{-1}$  concentrations of some trace metals to be analysed. Furthermore, the dissolved organic carbon (DOC) concentration is not required, as equilibrium between all solution components is achieved.



This technique differs from that proposed by Senn *et al* (2004), as the resin is in direct contact with the water, potentially allowing much quicker equilibration times than if a diffusive separation is employed.

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## **Appendix 1                      Sample pre-treatment for Chelex column studies**

Samples are generally subject to filtering as the first treatment step, most commonly with 0.45µm membrane filter thus preventing blockage of the column. Further pre-treatment of the sample will depend upon the nature of the study. For a study requiring a preconcentration step to determine total metal only, it is necessary to ensure that all metal exists in simple hydrated form or in Chelex-labile species and to buffer the sample to the optimum pH for Chelex sorption. This has been achieved by two methods. 1- The sample is acidified to strip trace elements bound by colloids (e.g. Greenberg & Kingston, 1983; and Vermeiren *et al*, 1990), care must be taken to neutralise or buffer the sample prior to flow-through to avoid poor sorption rates. 2- The sample is UV irradiated, this causes the oxidation of organic matter, thereby releasing organically bound trace metals (e.g. Florence & Batley, 1977). Moreover, Paulson (1986) reports that during extraction of previously acidified samples (neutralised before being passed through the column), organic material retained some capacity to inhibit Chelex trace metal sorption. In an effort to determine the best pre-treatment methods Paulson investigated various methods including using both acidification and UV treatment. The study concluded that the recovery was generally not increased in comparison to acidified-only treated samples at a flow rate of 0.2 ml min<sup>-1</sup>; however, the technique may be beneficial when using faster flow rates.

In the case of lability studies, the sample is generally filtered but otherwise untreated as acid or UV treatment will cause the Chelex-labile fraction to increase.

# Determination of Free Ion Trace Metal Concentrations in Freshwaters Using Cation-Exchange Resin Equilibrium. A Preliminary Investigation.

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**The dissolved free metal ion in natural waters is often related to bioavailability. However, no method currently exists which allows quick, simple and routine determination of free ion concentrations. This study aims to investigate the potential for an ion exchange resin (Chelex-100) to determine free ion concentrations by allowing the resin to equilibrate in situ with a natural water. The environmental concentrations can then subsequently be determined by back calculation of the concentrations eluted from the resin. The sampler employs resin beads retained between two layers of PE mesh, thus allowing flow through the device when deployed vertically in the flow of a stream. Metals are back extracted into a known volume of 2M HNO<sub>3</sub>. The Windermere Humic Aqueous model (WHAM6.1) was used to predict values for the equilibrium concentration of resin bound metals. Experimental Mn & Cd values were higher than predicted and Cu & Ni values were three orders of magnitude lower than predicted. However, these data are consistent with WHAM modelled data indicating the trend of resin-metal binding during a deployment. These data indicated that the deployment time was insufficient. The suitability of free ligand stability constants to resin binding and possible method developments are also discussed.**

Many trace metal species are observed in natural waters, the distribution between the possible forms being dependent upon the type and concentration of the trace metal, pH,  $E_h$  and types of organic and inorganic ligands, and colloidal surfaces (1). Furthermore, some metals have been shown to exhibit almost complete complexation in surface waters (e.g. Cu(II) in organic complex), whereas others (e.g. Mn(II)) show negligible organic complexation (2).

The bioavailability of metals in natural waters is often related to the dissolved free metal ( $M^{x+}$ ) concentration (3). Until the aspiration of a direct, in-situ and instantaneous free-ion activity analysis has been achieved, the principles of metal ion determination and the bioavailability of metals will continue to focus upon the distinction of the various species in which metal-ions exist. The use of a 0.45 $\mu$ m filter to operationally define the dissolved metal fraction of a natural water is commonplace. Methods such as anodic stripping voltammetry determine the very labile (~2ms) fraction, this includes very labile species such as Cu-glycine, as well as the free metal ions (4).

Techniques such as ion selective electrodes have been developed that do measure the free ion concentration (or more accurately activity) for some metals. However, they cannot presently be routinely operated in natural waters due to poor sensitivity.

Ion-exchange resins have been used for several decades for the preconcentration of metal ions from natural waters. One of the most widely used resins is Chelex-100 (stated from this point as Chelex) which utilises iminodiacetic acid (IDA) as the functional group. The amount of metal sorbed onto the resin is a function of the concentrations of the competing metals and ligands as well as the contact time of the sample with the resin and the binding capacity of the resin (stability constants of the metal-ligand species). Chelex has a large affinity for several metals that exist in trace concentrations in the environment. This affinity may be a result of the strong binding between nitrogen and the d block metals as well as the stability inferred by further binding to the two adjacent carboxylic groups. Smaller, more abundant ions such as sodium or magnesium tend only to bind to the carboxylic groups, thus making Chelex ideal for the separation of trace metals from bulk solutions. Of the resins studied by Wu and Lau (5) Chelex was found to be the most effective in taking up the soluble fraction of three metals studied (Cd, Pb and Zn) at low concentrations (0.1-10 mg l<sup>-1</sup>).

The main focus of Chelex use since the late 1970s has been the preconcentration of trace metals from seawater (6-9). Several speciation schemes have also been developed where Chelex separations form a key component (10-12). Over the past decade one of the major developments in the use of Chelex has been diffusive gradients in thin-films (DGT) (13). This technique allows trace metal concentrations to be calculated for freshwaters and seawaters as well as in soils (14) and sediments (15). However, all of these uses involve the accumulation of metal from labile species as well as unspicated metal ions. Furthermore, in these applications the stability constants of metal-resin complexes are not required as the resin binding sites are in sufficient excess to the metals that will be sorbed, so that competition effects are negligible.

A recent study by Senn et al (3) has investigated the potential of using an IDA resin (Toyopearl AF-Chelate 650M) to determine Cu<sup>2+</sup> concentrations by allowing the resin to equilibrate with the bulk solution. It comprises of a layer of polyacrylamide gel into which the resin beads are incorporated. The sampler was not deployed in a

natural water in this preliminary study, but was shown to be effective across a range of salinities and copper concentrations in synthetic solutions. However, long equilibration times are required (12 days). The other drawback of this technique is the need to substitute Chelex stability constants with those of free IDA, this may not be appropriate as resin-IDA binding may not be equal to IDA binding.

The work presented here aims to investigate the potential for Chelex equilibrium to determine free-ion concentrations in natural waters. The method uses Chelex beads in direct contact with water in situ and may allow back calculation of the natural water metal concentrations from bound metals eluted from the resin with acid. The objectives of this preliminary study are to establish the deployment time required and to investigate the suitability of the IDA or methyl-IDA (MIDA) stability constants to Chelex binding. This will be achieved by comparing experimentally determined concentrations with values predicted by the speciation model Windermere Humic Aqueous Model version 6.1 (WHAM6.1) (16).

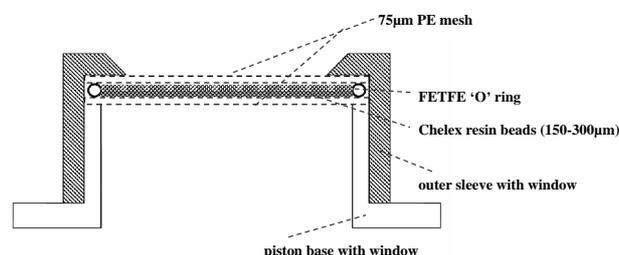
## EXPERIMENTAL

**Reagents.** Analytical grade Chelex-100 (Na form, 100-200 mesh) was obtained from Bio-Rad Laboratories (batch number 58544B). As the water content of the resin varies between 68-76% (17) the number of IDA groups within a given mass of resin cannot be determined accurately by its weight. However, as 1ml of Na-form resin slurry contains 0.2 mmol (0.4 meq) of binding sites an accurate measurement can be made of the IDA groups within a given mass of resin. This was done by making a slurry of 2g of resin in a measuring cylinder using MQ water. The volume of resin can be directly observed after the beads have been allowed to settle. The IDA concentration of one gram of resin was found to be 0.31 mmol (0.61 meq).

The acid used for elution of trace metals from the resin and washing of the deployment units was J. T. Baker ULTREX II Ultrapure reagent grade nitric acid (lot number A35439).

**Chelex Sampler Setup.** The Chelex equilibrium sampler (CES) uses a modified DGT solution deployment unit (DGT Research Ltd, Lancaster). The piston base was drilled to give a window allowing flow through the device. A known mass (approximately 50 mg) of Chelex resin was contained by two layers of 75 $\mu$ m PE mesh and an FETFE O-ring. This is held together by the outer sleeve and piston base of the deployment unit. Blanks were also prepared in the same way, with the Chelex excluded. The sampler is illustrated in Figure 1.

**Sampler Deployment.** Prepared deployment units were acid washed in 2M HNO<sub>3</sub> acid for a period of 30 minutes immediately prior to deployment, followed by two rinsing steps with MQ water. This acid wash was undertaken directly before deployment due to the poor chemical exchange properties that may occur after the resin has been in the H<sup>+</sup> form for longer than a few hours (17). Triplicate Chelex samplers were deployed for each time interval together with one blank. Deployments were undertaken for 1, 3, 6 and 24 hours.



**Figure 1.** Representation of the Chelex equilibrium sampler.

The sample site is a slow flowing stream tributary of the River Conder, situated at Hazelrigg, Lancaster. Samplers were deployed vertically in the flow of the stream within a holder that incorporated a layer of mesh to prevent the samplers becoming soiled with silt and debris. On retrieval the samplers were immediately rinsed with MQ water to wash off any particulate and to prevent further accumulation of trace metals, then placed in a clean plastic bag until return to the lab for analysis.

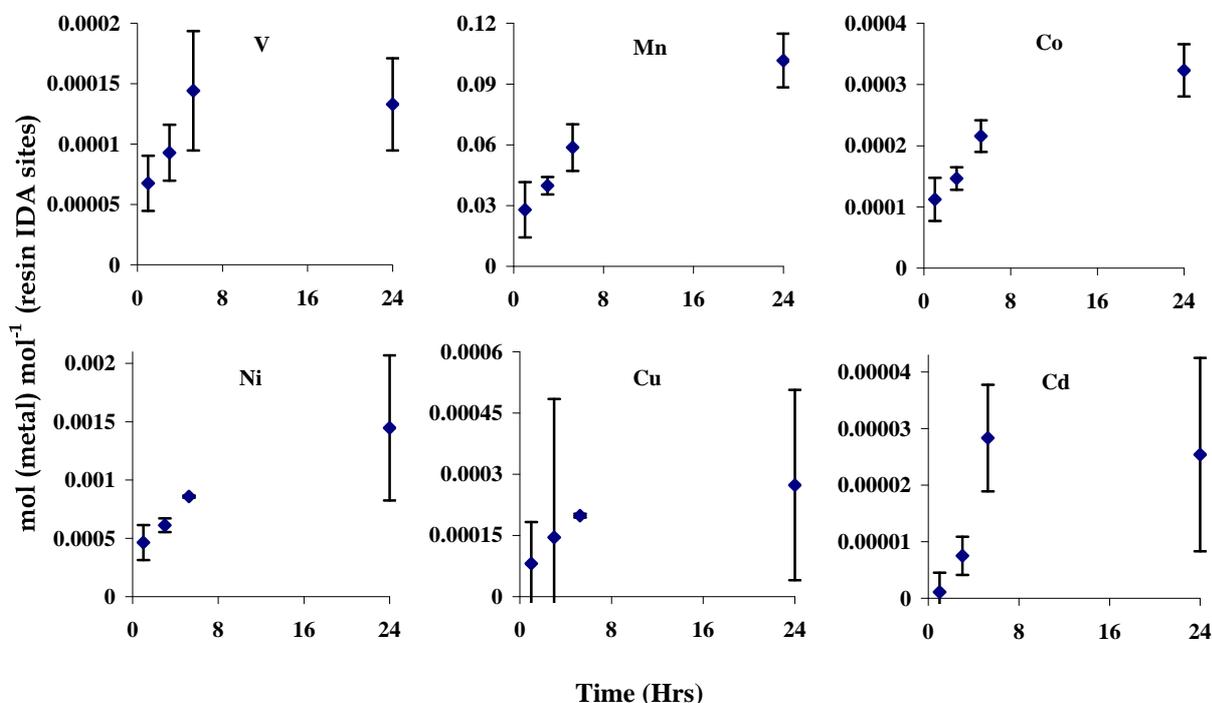
All handling in the laboratory was undertaken in a laminar flow cabinet. Samplers were given a further rinse with MQ water before the entire unit was immersed in 25ml of 2M HNO<sub>3</sub> acid for 30 minutes to elute the trace metals, the vessels were shaken at five minute intervals to ensure efficient elution. Trace metal analysis was performed using inductively coupled plasma mass spectrometry (ICPMS).

## RESULTS AND DISCUSSION

**Resin Uptake Over 24 Hour Deployments.** Figure 2 shows the concentration of metal sorbed to Chelex with time, expressed as moles of metal per mole of Chelex IDA groups. Sorption of all metals is rapid over the first six hours, this is followed by a lesser increase up to the 24 hour deployment. In the case of V and Cd a decrease in sorbed concentrations may occur in the 6-24hr period. The initial uptake of metal will correspond with an exchange of metals with H<sup>+</sup> as the resin is deployed in the H<sup>+</sup> form. Significant Ca sorption is expected during the deployment period employed in this study as Ca is present at the deployment site at concentrations an order of magnitude higher than Mn and the selectivity of Chelex for Ca is reported to be only half that of Mn (17).

Given the very large selectivity of Chelex for trace metals, particularly Cu, together with the trends of increasing concentration over time shown in Figure 2, it is unlikely that the system has reached equilibrium.

**Comparison of Experimental vs. Modelled Values.** To predict if the metal values determined experimentally represented the equilibrium values the WHAM6.1 speciation model (16) was used to determine the concentrations of trace metals bound to the resin when in equilibrium with the stream water. This was done by using both IDA and MIDA stability constants (18-21) to observe the difference that an additional non-functional group (methyl in this case) has on the equilibrium values. Assessing IDA constants only, does not allow for consideration of the effect of the polymer matrix on the Chelex IDA groups.



**Figure 2.** Concentration of metal bound per mole of Chelex IDA sites with time over a 24 hour deployment. Error bars represent two standard deviations from the mean except Cu and Cd which are one standard deviation. Values are based on triplicate analyses except the six hour data, which is based on two replicates due to the spoiling of one sample.

Table I shows the experimental values and the modelled equilibrium values calculated by WHAM6.1. These data show that the metal values from the stream deployments are significantly different from those predicted, Mn concentrations are significantly larger and Cu & Ni values are much lower than predicted. This supports the assertion that the 24 hour deployment is not at equilibrium with the stream water.

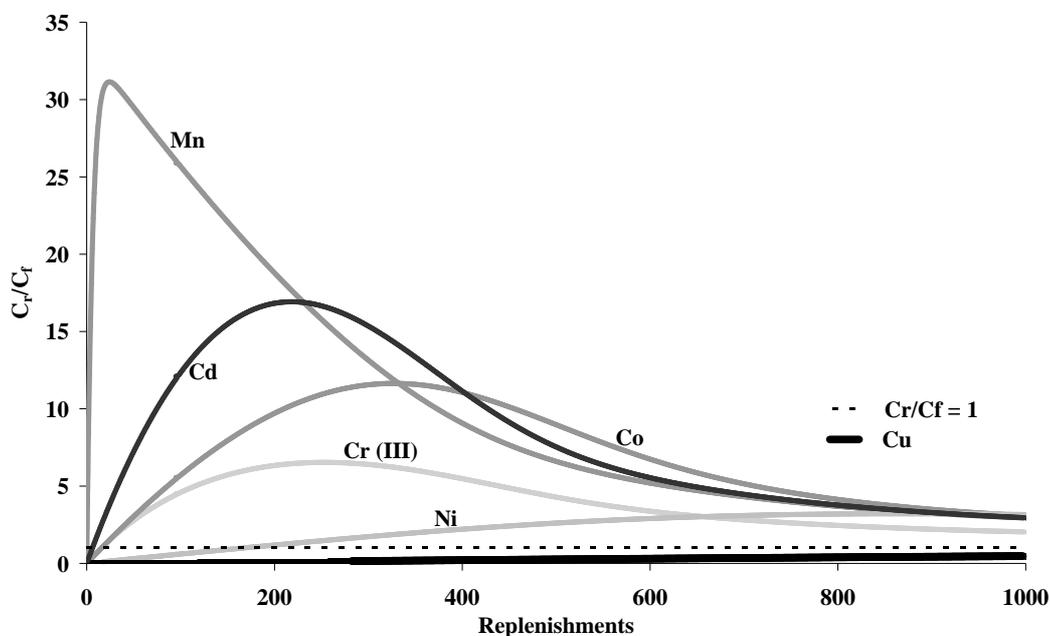
**Modelled Behaviour of Chelex Using MIDA Stability Constants.** To determine the theoretical behaviour of Chelex under deployment, a model was set up which predicted the thermodynamic behaviour of the resin over time during a deployment. The model incorporated WHAM6.1 to determine output values and Excel to determine input values. The model assumed 50 mg of resin was in contact with 5ml of water at any one time, this equates to a solution of 3 mM MIDA. MIDA stability constants were used as a greater number of published values were available than for IDA. Trace metal values for the model input were obtained from ICPMS analysis of the stream water. Model inputs also included dissolved organic carbon,  $p\text{CO}_2$  and estimated values for major cations & anions. The model allowed the resin to equilibrate with an aliquot of sample, the concentration of selected trace metals (Cr, Mn, Co, Ni,

Cu, Zn & Cd) bound to the resin was then added to the initial concentration of metal, effectively representing the addition of the resin to another aliquot of sample (a replenishment). This process was repeated until the modelled free ion concentration of all metals was equal to the values calculated from the sample without the Chelex present. This stage represents equilibrium between the resin and the sample.

Figure 3 shows the modelled behaviour of Chelex in the environment described above. The concentration bound at a given replenishment divided by the predicted concentration bound at equilibrium, is plotted against the number of replenishments. Equilibrium is reached at approximately 2000 replenishments (not shown in plot). It is evident that due to low concentration of Cu in the water the resin binding sites are initially occupied by more abundant but less chemically attractive metals. As more sample contacts the resin the more competitive Cu replaces the other metals. The overall effect is to overestimate the concentrations of the majority of trace metals if a Chelex sampler is not deployed for a sufficient period of time. This could account for the higher than predicted values found for Mn and Cd for the 24hr deployment.

**Table I. Experimental values for 24hr sampler deployment and modelled equilibrium values calculated by WHAM6.1. Units: mol (metal) mol<sup>-1</sup> (resin IDA sites).**

Metal		Cr	Mn	Co	Ni	Cu	Zn	Cd
Predicted	IDA	$3.7 \times 10^{-3}$	$4.9 \times 10^{-3}$	$1.5 \times 10^{-3}$	0.14	0.78	0.019	$3.4 \times 10^{-6}$
	MIDA	$1.8 \times 10^{-3}$	$7.5 \times 10^{-3}$	$2.4 \times 10^{-3}$	0.14	0.80	0.024	$2.2 \times 10^{-5}$
Experimental 24hr		—	0.12	$3.2 \times 10^{-4}$	$1.5 \times 10^{-4}$	$2.7 \times 10^{-4}$	—	$2.5 \times 10^{-5}$



**Figure 3.** Modelled behaviour of MIDA. The ratio of the actual metal concentration and the expected concentration at equilibrium is plotted against the number of replenishments. See text for discussion.

The values for Cu and Ni from the 24hr deployments are approximately three orders of magnitude less than those predicted, suggesting that the data from the experimental deployment lay to the extreme left of the plot in Figure 3. Furthermore, the model does not account for kinetic factors and assumes that all exchange occurs instantaneously, this is unlikely to be the case in the real system. A significant fraction of the metals may be bound in species with slow exchange kinetics extending the time required before equilibrium is achieved.

**Suitability of IDA or MIDA constants to Chelex-IDA behaviour.** Given that the experimental deployments have been shown not to have reached equilibrium, these data cannot be used to test the suitability of IDA and MIDA stability constants. However, several studies have sought to investigate Chelex stability constants and examine the binding nature of the resin compared to free IDA/MIDA binding.

Loewenschuss & Schmuckler (22) reported stability constants for Dowex (a resin very similar to Chelex) Cu & Ni complexes which indicate that resin binding of these two metals is much greater than binding with the equivalent free ligand. Cu is reported to have a log stability constant of 16.9 for Dowex compared to the a value of 10.55 for the free ligand in solution and the Dowex Ni value is 15.6 compared to 8.21 for the free ligand. Furthermore, Bayer (23) had previously highlighted that certain chelating ligands bound to macromolecules have a higher selectivity for selected metal ions than the same mono-ligand because of the steric arrangement of sites in the macromolecule. Loewenschuss & Schmuckler concluded that this was not the case for Dowex. However, a recent study by Atzei et al (24) concluded that the low degree of polymerisation (low cross linkage) in Chelex can allow the favourable orientation of the IDA groups, enabling the metal to coordinate with two or more ligand groups.

The small but significant difference in the predicted equilibrium concentrations of Cu and Zn using the IDA and MIDA stability constants (reported in Table I) provides additional evidence that larger functional groups may affect the selectivity of the chelating ligand for certain trace metals.

**Method Development.** One of the principal drawbacks of the method used in this study was the poor flow rate through the device, due to the need to incorporate a pre-filter to avoid fouling of the sampler. This had the effect of diverting some flow around the holder decreasing the water passing through the device. A more robust holder possibly with a larger mesh filter may allow the device to be deployed in streams with a more significant flow. There is also a significant possibility that regardless of the flow rate the binding of trace metals onto binding sites within the resin beads will be regulated by the rate of diffusion into the polymer matrix. Use of the smaller Chelex beads (75-150 $\mu$ m) may overcome this problem; although the mesh in the sampler would also have to be finer (63 $\mu$ m should retain this bead size).

Similar methods which have been tested but involve diffusion processes requiring long equilibration times, include use of diffusive gel (12 days) (3) and a method where vialing tubing is employed (up to 21 days) (5). Neither method has been tested in natural waters. Consequently, the principle of allowing binding sites to directly contact the environment of interest is likely to provide a more practical method than diffusion based techniques, providing a means of achieving quick ( $\leq$  24hr) equilibration can be developed.

The use of an alternative resin may also improve the equilibration time. An ideal resin will have an open structure with sufficient cross linkage to give a rigid structure, i.e. not allowing shrinkage or multiple coordination. It must contain sufficient chelating ligands to achieve adequate metal concentrations for analysis and

must also have a bead size large enough to be retained by a mesh which allows adequate flow.

The possibility of introducing Chelex into a water sample brought to the lab, which may enable efficient mechanical mixing, is limited by the large volumes of water that would be required. To equilibrate 30 mg of resin with sufficient water so that no significant reduction in trace metals occurs, requires approximately 100l of sample (estimated using the stream water values from this study and WHAM6.1). Furthermore large fractions of organically speciated trace metal may further complicate analysis by increasing the volumes required.

### CONCLUSIONS

This study has determined that the deployment time for the employed setup was insufficient and this conclusion was supported by both the equilibrium values and the deployment trend concentrations determined by WHAM6.1.

The use of Chelex/natural water equilibration has the ability to provide a routine analysis method for trace metals. However, the technique will require development in order that deployment times can be minimised. Furthermore, stability constants for the resin must be determined as IDA or MIDA constants are not a suitable substitute.

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